

What is claimed:

1. A plasmid comprising an rRNA gene having a mutant Anti-Shine-Dalgarno
5 sequence, at least one mutation in said rRNA gene, and a genetically engineered gene
which encodes a selectable marker having a mutant Shine-Dalgarno sequence, wherein
the mutant Anti-Shine-Dalgarno and the mutant Shine-Dalgarno sequence are a mutually
compatible pair.
- 10 2. The plasmid of claim 1, wherein the rRNA gene is from a species selected from
the group consisting of *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*,
Salmonella typhi, *Yersenia pestis*, *Staphylococcus aureus*, *Streptococcus pyogenes*,
Enterococcus faecalis, *Chlamydia trachomatis*, *Saccharomyces cerevesiae*, *Candida*
albicans, and *trypanosome*.
- 15 3. The plasmid of claim 1, wherein the selectable marker is chosen from the group
consisting of chloramphenicol acetyltransferase (CAT), green fluorescent protein (GFP),
and both CAT and GFP.
- 20 4. The plasmid of claim 1, wherein the mutant Anti-Shine-Dalgarno sequence is
selected from the group consisting of the sequences set forth in Figures 12, 13, 15, and
16.
5. The plasmid of claim 1, wherein the mutant Shine-Dalgarno sequence is selected
25 from the group consisting of the sequences set forth in Figures 12, 13, 15, and 16.
6. The plasmid of claim 1, wherein the mutant Anti-Shine-Dalgarno sequence and
the mutant SD sequence are a mutually compatible pair selected from the group
consisting of the sequences set forth in Figures 12, 13, 15, and 16.
- 30 7. The plasmid of claim 6, wherein the mutually compatible mutant Shine-Dalgarno
and mutant Anti-Shine-Dalgarno pair permits translation by the rRNA of the selectable
marker.
- 35 8. The plasmid of claim 3, wherein the selectable marker is CAT.
9. The plasmid of claim 3, wherein the selectable marker is GFP.

10. A cell comprising the plasmid of claim 1.

11. The cell of claim 10, wherein the mutations in the rRNA gene affect the quantity of selectable marker produced.

12. The cell of claim 10, wherein the cell is a bacterial cell.

13. The plasmid of claim 1, wherein the DNA sequence encoding the rRNA gene is under the control of an inducible promoter.

14. A plasmid comprising an *E. coli* 16S rRNA gene having a mutant Anti-Shine-Dalgarno sequence, at least one mutation in said 16S rRNA gene, and a genetically engineered gene which encodes GFP having a mutant Shine-Dalgarno sequence, wherein the mutant Anti-Shine-Dalgarno and the mutant Shine-Dalgarno sequence are a mutually compatible pair.

15. The plasmid of claim 14, wherein the mutant Anti-Shine-Dalgarno sequence is selected from the group consisting of the sequences set forth in Figures 12, 13, 15; and 16.

16. The plasmid of claim 14, wherein the mutant Shine-Dalgarno sequence is selected from the group consisting of the sequences set forth in Figures 12, 13, 15, and 16.

17. The plasmid of claim 14, wherein the mutant Anti-Shine-Dalgarno sequence and the mutant Shine-Dalgarno sequence are a mutually compatible pair selected from the group consisting of the sequences set forth in Figures 12, 13, 15, and 16.

18. The plasmid of claim 17, wherein the mutually compatible mutant Shine-Dalgarno and mutant Anti-Shine-Dalgarno pair permits translation by the mutant 16S rRNA of the selectable marker GFP.

19. A cell comprising the plasmid of claim 14.

20. The cell of claim 19, wherein the mutation in the 16S rRNA gene affects the quantity of selectable marker produced.

21. The cell of claim 19, wherein the cell is a bacterial cell.

22. The plasmid of claim 14, wherein the DNA sequence encoding the 16S rRNA gene is under the control of an inducible promoter.

5 23. A method for identifying functional mutant ribosomes comprising:

- (a) transforming a host cell with a plasmid comprising an rRNA gene having a mutant Anti-Shine-Dalgarno sequence, at least one mutation in said rRNA gene, and a genetically engineered gene which encodes a selectable marker having a mutant Shine-Dalgarno sequence, wherein the mutant Anti-Shine-Dalgarno and the mutant Shine-Dalgarno sequence are a mutually compatible pair;
- (b) isolating cells via the selectable marker; and
- (c) identifying the rRNA from the cells from step (b), thereby identifying functional mutant ribosomes.

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24. A method for identifying functional mutant ribosomes comprising:

- (a) transforming a host cell with a plasmid comprising an *E. coli* 16S rRNA gene having a mutant Anti-Shine-Dalgarno sequence, at least one mutation in said 16S rRNA gene, and a genetically engineered gene which encodes GFP having a mutant Shine-Dalgarno sequence, wherein the mutant Anti-Shine-Dalgarno and the mutant Shine-Dalgarno sequence are a mutually compatible pair;
- (b) isolating cells via the GFP; and
- (c) identifying the rRNA from the cells from step (b), thereby identifying functional mutant ribosomes.

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25. A method for identifying functional mutant ribosomes that may be suitable as drug targets comprising:

- (a) transforming a host cell with a plasmid comprising an rRNA gene having a mutant Anti-Shine-Dalgarno sequence, at least one mutation in said rRNA gene, and a genetically engineered gene which encodes a selectable marker having a mutant Shine-Dalgarno sequence, wherein the mutant Anti-Shine-Dalgarno and the mutant Shine-Dalgarno sequence are a mutually compatible pair;
- (b) isolating cells via the selectable marker;
- (c) identifying and sequencing the rRNA from the cells from step (b), thereby identifying regions of interest;
- (d) selecting regions of interest from step (c);

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- (e) mutating the regions of interest of step (d);
- (f) inserting the mutated regions of interest from step (e) into a plasmid comprising an rRNA gene having a mutant Anti-Shine-Dalgarno sequence and a genetically engineered gene which encodes a selectable marker having a mutant Shine-Dalgarno sequence, wherein the mutant Anti-Shine-Dalgarno and the mutant Shine-Dalgarno sequence are a mutually compatible pair;
- (g) transforming a host cell with the plasmid from step (f);
- (h) isolating cells of step (g) via the selectable marker; and
- (i) identifying the rRNA from step (h), thereby identifying functional mutant ribosomes that may be suitable as drug targets.

26. A method for identifying functional mutant ribosomes that may be suitable as drug targets comprising:

- (a) transforming a host cell with a plasmid comprising an *E. coli* 16S rRNA gene having a mutant Anti-Shine-Dalgarno sequence, at least one mutation in said 16S rRNA gene, and a genetically engineered gene which encodes GFP having a mutant Shine-Dalgarno sequence, wherein the mutant Anti-Shine-Dalgarno and the mutant Shine-Dalgarno sequence are a mutually compatible pair;
- (b) isolating cells via the GFP;
- (c) identifying and sequencing the rRNA from the cells from step (b), thereby identifying regions of interest;
- (d) selecting the regions of interest from step (c);
- (e) mutating the regions of interest from step (d);
- (f) inserting the mutated regions of interest from step (e) into a plasmid comprising an *E. coli* 16S rRNA gene having a mutant Anti-Shine-Dalgarno sequence and a genetically engineered gene which encodes GFP having a mutant Shine-Dalgarno sequence, wherein the mutant Anti-Shine-Dalgarno and the mutant Shine-Dalgarno sequence are a mutually compatible pair;
- (g) transforming a host cell with the plasmid from step (f);
- (h) isolating cells of step (g) via the GFP; and
- (i) identifying the rRNA from step (h), thereby identifying functional mutant ribosomes that may be suitable as drug targets.

27. A method for identifying drug candidates comprising:

- (a) transforming a host cell with the plasmid of claim 1;

- (b) isolating cells via the selectable marker;
- (c) identifying and sequencing the rRNA from step (b) to identify the regions of interest;
- (d) selecting regions of interest from step (c);
- 5: (e) mutating the regions of interest from step (d);
- (f) inserting the mutated regions of interest from step (e) into a plasmid comprising an rRNA gene having a mutant Anti-Shine-Dalgarno sequence and a genetically engineered gene which encodes a selectable marker having a mutant Shine-Dalgarno sequence, wherein the mutant Anti-Shine-Dalgarno and the mutant Shine-Dalgarno sequence are a mutually compatible pair;
- 10 (g) transforming a host cell with the plasmid from step (f);
- (h) isolating cells from step (g) via the selectable marker;
- (i) identifying the rRNA from step (h) to identify the functional mutant ribosomes;
- 15 (j) screening drug candidates against functional mutant ribosomes from step (i);
- (k) identifying the drug candidates from step (j) that bound to the functional mutant ribosomes from step (i);
- 20 (l) screening the drug candidates from step (k) against a human rRNA; and
- (m) identifying the drug candidates from step (l) that do not bind to the human rRNA, thereby identifying drug candidates.

28. A method for identifying drug candidates comprising:

- 25 (a) transforming a host cell with the plasmid of claim 14;
- (b) isolating cells via the selectable marker;
- (c) identifying and sequencing the rRNA from step (b) to identify the regions of interest;
- (d) selecting the regions of interest from step (c);
- 30 (e) mutating the regions of interest from step (d);
- (f) inserting the mutated regions of interest from step (e) into a plasmid comprising an *E. coli* 16S rRNA gene having a mutant Anti-Shine-Dalgarno sequence and a genetically engineered gene which encodes GFP having a mutant Shine-Dalgarno sequence, wherein the mutant Anti-Shine-Dalgarno and the mutant Shine-Dalgarno sequence are a mutually compatible pair;
- 35 (g) transforming a host cell with the plasmid from step (f);
- (h) isolating cells from step (g) via the selectable marker;

- (i) identifying the rRNA from step (h) to identify the functional mutant ribosomes;
- (j) screening drug candidates against the functional mutant ribosomes from step (i);
- 5 (k) identifying the drug candidates from step (j) that bound to the functional mutant ribosomes from step (i);
- (l) screening the drug candidates from step (k) against a human 16S rRNA; and
- 10 (m) identifying the drug candidates from step (l) that do not bind to the human 16S rRNA, thereby identifying drug candidates.